PTX animals increased FGF23 and calcitriol and normalized serum phosphate levels. Several observations had already suggested that PTH could stimulate skeletal FGF23 production. Administration of (1-34)PTH to healthy individuals increased serum FGF23. Moreover, constitutively activated PTHR1 in Jansen's disease, which can be considered as a primary hyperparathyroidism with high PTH and hypophosphatemia, showed elevated FGF23.[9] Similarly, high FGF23 is often seen in secondary hyperparathyroidism and can predict its severity and its resistance to vitamin D therapy in chronic kidney disease patients.[10] In the same patients, serum FGF23 significantly declined after parathyroidectomy. López *et al.*[8] confirm and extend these previous findings, demonstrating that PTH, either directly or indirectly via calcitriol, is necessary for skeletal FGF23 production.

Probably, one of the most interesting findings of this study is the lack of effect of FGF23 on urinary and serum phosphate in the absence of PTH. PTX animals receiving physiological doses of calcitriol had high FGF23 but did not normalize serum phosphate, implying that, at least in the kidney, there was a key factor regulated by PTH that was required for the phosphaturic action of FGF23. This factor was certainly klotho, as the authors found a reduction of klotho expression in the kidneys of PTX animals. Importantly, renal klotho expression was restored to normal levels after PTH supplementation, allowing FGF23 to exert its phosphaturic action and the normalization of serum phosphate levels.

In summary, the study by López *et al.*[8] shows that PTH is necessary in maintaining normal circulating FGF23 levels, that changes in calcitriol levels unquestionably play a role in the stimulatory effect of PTH on FGF23, and that PTH regulates klotho expression in the kidney and thereby the phosphaturic effect of FGF23. It confirms the existence of three feedback loops, involving parathyroid glands, bone, and kidney, regulating serum phosphate concentration (Figure 1) and opens a number of areas for clinical and experimental research in the field of phosphate homeostasis.

DISCLOSURE

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REFERENCES

1. Potts JT. Parathyroid hormone: past and present. *J Endocrinol* 2005; **187**: 311–325.
2. Shimada T, Mizutani S, Muto T *et al.* Cloning and characterization of FGF23 as a causative factor of tumor-induced osteomalacia. *Proc Natl Acad Sci USA* 2001; **98**: 6500–6505.
3. Kuro-o M, Matsumura Y, Aizawa H *et al.* Mutation of the mouse klotho gene leads to a syndrome resembling ageing. *Nature* 1997; **390**: 45–51.
4. Hu MC, Shi M, Zhang J *et al.* Klotho: a novel phosphaturic substance acting as an autocrine enzyme in the renal proximal tubule. *FASEB J* 2010; **24**: 3438–3450.
5. Krajisnik T, Bjorklund P, Marsell R *et al.* Fibroblast growth factor-23 regulates parathyroid hormone and 1alpha-hydroxylase expression in cultured bovine parathyroid cells. *J Endocrinol* 2007; **195**: 125–131.
6. Ichikawa S, Imel EA, Kreiter ML *et al.* A homozygous missense mutation in human KLOTHO causes severe tumoral calcinosis. *J Clin Invest* 2007; **117**: 2684–2691.
7. Imura A, Tsuji Y, Murata M *et al.* α-Klotho as a regulator of calcium homeostasis. *Science* 2007; **316**: 1615–1618.
8. López I, Rodríguez-Ortiz ME, Almadén Y *et al.* Direct and indirect effects of parathyroid hormone on circulating levels of fibroblast growth factor 23 *in vivo*. *Kidney Int* 2011; **80**: 475–482.
9. Brown WW, Juppner H, Langman CB *et al.* Hypophosphatemia with elevations in serum fibroblast growth factor 23 in a child with Jansen's metaphyseal chondrodysplasia. *J Clin Endocrinol Metab* 2009; **94**: 17–20.
10. Shigematsu T, Kazama JJ, Yamashita T *et al.* Possible involvement of circulating fibroblast growth factor 23 in the development of secondary hyperparathyroidism associated with renal insufficiency. *Am J Kidney Dis* 2004; **44**: 250–256.

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Calcium sensing in podocytes

Maria P. Rastaldi[1]

Besides its primary function in maintaining systemic calcium homeostasis, the calcium-sensing receptor (CaSR) is expressed by many cell types, with different, sometimes opposite, regulatory functions. Novel work from Oh and collaborators shows that activation of CaSR in podocytes has prosurvival effects and protects the cell from puromycin aminonucleoside damage. Given that the cellular consequences of CaSR activation are largely context-dependent, further studies will be required to elucidate its precise role in podocyte physiology and pathophysiology.

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Since 1883, when Ringer discovered that a trace amount of Ca^{2+} from tap water was sufficient to induce contraction of a frog's heart, the role of Ca^{2+} in regulating physiological functions has been continually investigated. We now know that appropriate concentrations of extracellular and intracellular Ca^{2+} are vital to the survival of all organisms from the simplest unicellular organism to mammals and that highly regulated processes are required to provide constant and appropriate quantities of the ion to cells and tissues. Therefore, sophisticated mechanisms enable cells to detect minor changes in extracellular Ca^{2+} content to counteract and modify their behavior accordingly.

In major organisms, Ca^{2+} has to be controlled both systemically and locally, and in mammals, the control of systemic calcium homeostasis is mediated by the calcium-sensing receptor (CaSR).

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First cloned from the parathyroid and the kidney, CaSR is a G protein-coupled receptor encoded by one gene, located in humans on chromosome 3. Its non-redundant role in maintaining appropriate levels of systemic calcium is well demonstrated by human diseases due to *CaSR* mutations; heterozygous and homozygous *CaSR*-inactivating mutations cause familial hypocalciuric hypercalcemia and neonatal severe hyperparathyroidism, respectively. On the other hand, gain-of-function mutation of *CaSR* leads to autosomal dominant hypoparathyroidism.

CaSR can be activated by a series of agonists that are classified, depending on their action, into type I and type II. Type I agonists, or true agonists, are divalent cations (namely, Ca^{2+} and Mg^{2+}), polycations (such as gadolinium, polyarginine, polylysine, and neomycin), and β-amyloid peptides, which directly activate the receptor. Type II agonists are better referred to as modulatory substances, because they allosterically increase the receptor's calcium affinity, thereby requiring extracellular calcium for their action. Physiological type II agonists are, for instance, spermine, spermidine, aromatic amino acid residues, extracellular pH, and ionic strength, meaning that activation of CaSR has to be considered always in the context of the cell microenvironment. Calcimimetics belong to the type II agonist family; they are positive allosteric CaSR modulators, currently in use for the treatment of hyperparathyroidism secondary to renal failure. Finally, the receptor can be allosterically negatively modulated by so-called calcilytics, of potential utility to treat osteoporosis.

After the first expression analyses conducted on parathyroid gland and kidney, the presence of CaSR has been demonstrated in several tissues and cells not related to systemic calcium homeostasis, and its function has been largely investigated, revealing that the receptor regulates a series of diverse, sometimes opposite, cellular functions.

If downstream effects can be highly different, the first steps of receptor activation are common; stimulation of the receptor evokes an increase in free ionized intracellular Ca^{2+} concentration, either through phospholipase C-dependent activation of inositol trisphosphate-sensitive stores or through activation of Ca^{2+}-sensitive cation channels.

To list some examples, the receptor has been implicated in gastrin and gastric acid secretion, keratinocyte differentiation, promotion or prevention of tumor growth (depending on the type of cancer), and insulin secretion from pancreatic islet β-cells, among others. CaSR has also been linked to inflammatory states, both with an elevation in cytokine production upon its stimulation, and with an upregulation of its expression when exposed to an inflammatory environment.

From all these studies in different systems a common concept emerges: the effects of CaSR activation are largely context-dependent, because the cellular environment has profound influences on its responses to ligands. This behavior is common for G protein-coupled receptors and is called 'conditional efficacy,' the ability of a receptor to sense not only the direct ligand but also the environment and to qualitatively and quantitatively adapt the answer(s) accordingly.

The presence of CaSR in the glomerulus is still under debate. The first evidence of a glomerular mRNA transcript of CaSR was shown in the rat kidney by Riccardi *et al.* in 1996 (ref. 1) but was not confirmed by Yang *et al.* in 1997 (ref. 2), and in a recent review, Riccardi *et al.* show negative CaSR immunostaining in human glomeruli.[3] In 2005 Kwak *et al.* studied CaSR expression and activity in mesangial cells,[4] and in 2008 the presence of the receptor on podocytes was shown for the first time, by *in situ* hybridization and immunohistochemistry, on rat renal tissue by Piecha *et al.*[5]

Now, the same group from Heidelberg (Oh *et al.*,[6] this issue) proceeds into the investigation and analyzes the potential role of CaSR in podocytes by a series of *in vitro* and *in vivo* studies aimed at clarifying some of the intracellular pathways activated by the receptor.

The authors show that CaSR stimulation by the calcimimetic R-568 of normal podocytes activates a prosurvival pathway (Figure 1), by inducing ERK1/2 phosphorylation and subsequent CREB phosphorylation. They also describe other effects, all in the same prosurvival direction, such as activation of pBAD and Bcl-xL, increase of Rho kinase activity, and decrease of cyclic adenosine monophosphate content. According to these data, activation of these intracellular pathways explains the protective effect exerted by R-568, which reduces apoptosis in podocytes incubated with puromycin aminonucleoside (PAN) and ameliorates podocyte cytoskeleton when damage is induced by either PAN or cytochalasin. Subsequently, the authors observe *in vivo* protecting effects of R-568 against PAN nephropathy, which occur when the molecule is administered either before or 2–4 days after PAN injection.

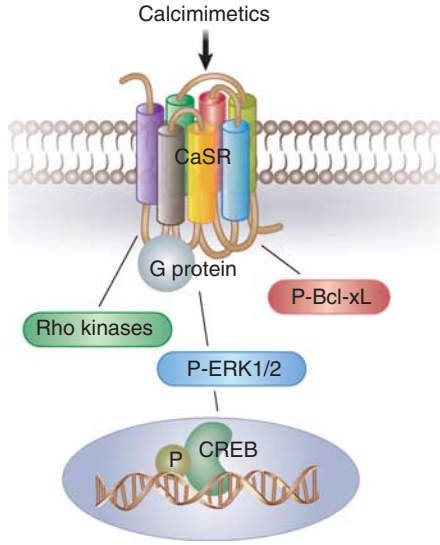

Figure 1 | The intracellular pathways activated in healthy podocytes upon stimulation of CaSR by calcimimetics, according to the results of Oh *et al.*[6] CaSR, calcium-sensing receptor.

There is no doubt that podocytes need tight calcium control. Mutations in the *TRPC6* calcium channel (transient receptor potential channel 6) gene have been associated with familiar forms of focal and segmental glomerulosclerosis. In addition, acquired glomerular diseases, especially membranous nephropathy, are associated with increased expression levels of TRPC6.[7] Our group has recently shown that podocytes express another calcium channel, previously thought to belong only to neuronal cells, the ionotropic *N*-methyl-D-aspartate (NMDA) receptor, whose blockade causes podocyte cytoskeletal damage and increased proteinuria.[8]

Interestingly, CaSR is also expressed by neuronal cells and is largely distributed in

several areas of the brain and cerebellum, where it overlaps with group I metabotropic glutamate receptors, especially Grm1. This proximity between CaSR and Grm1 has been analyzed, because amino acids and calcium reciprocally influence both receptors. Heterodimers of CaSR and Grm1 have also been demonstrated that can lead to signal enhancement, changes in sensitivity to agonists, and differences in trafficking of the receptors themselves.

Our group has described the use by podocytes of a neuron-like system of signaling,[9] and we have recently observed that mice lacking one or both alleles of Grm1 have podocyte alterations and higher proteinuria than the corresponding wild type.[10] In podocytes, Grm1 colocalizes with nephrin, and its silencing causes disappearance of nephrin from podocyte processes and alters podocyte actin cytoskeleton. In view of these data, the relationship and the potential reciprocal influences of CaSR and Grm1 at the glomerular filtration barrier could be worth studying.

Given the context-dependent nature of CaSR, the promising experimental results obtained by Oh *et al.*[6] cannot be easily generalized, and experiments on primary podocytes and on different experimental models will be required before better knowledge can be gained on the role of the receptor in the glomerulus. An inducible podocyte-specific CaSR knockout model would be ideal to shed further light on the activity of the receptor at the glomerular filtration barrier.

The potential CaSR expression by mesangial cells needs further investigation as well. Previous data showed that activation of CaSR induces mesangial-cell proliferation, certainly not a desirable effect in most human glomerular disease settings.

In summary, the data published by Oh *et al.* open an intriguing chapter of investigation on podocyte biology that may extend our knowledge of the mechanisms used by this cell to control its intracellular calcium content and to properly respond to microenvironmental changes.

DISCLOSURE
The author declared no competing interests.

REFERENCES

1. Riccardi D, Lee WS, Lee K *et al.* Localization of the extracellular Ca(2+)-sensing receptor and PTH/PTHrP receptor in rat kidney. *Am J Physiol* 1996; **271**: F951–F956.
2. Yang T, Hassan S, Huang YG *et al.* Expression of PTHrP, PTH/PTHrP receptor, and Ca(2+)-sensing receptor mRNAs along the rat nephron. *Am J Physiol* 1997; **272**: F751–F758.
3. Riccardi D, Finney BA, Wilkinson WJ, Kemp PJ. Novel regulatory aspects of the extracellular Ca^{2+}-sensing receptor, CaR. *Pflugers Arch* 2009; **458**: 1007–1022.
4. Kwak JO, Kwak J, Kim HW *et al.* The extracellular calcium sensing receptor is expressed in mouse mesangial cells and modulates cell proliferation. *Exp Mol Med* 2005; **37**: 457–465.
5. Piecha G, Kokeny G, Nakagawa K *et al.* Calcimimetic R-568 or calcitriol: equally beneficial on progression of renal damage in subtotally nephrectomized rats. *Am J Physiol Renal Physiol* 2008; **294**: F748–F757.
6. Oh J, Beckmann J, Bloch J *et al.* Stimulation of the calcium-sensing receptor stabilizes the podocyte cytoskeleton, improves cell survival, and reduces toxin-induced glomerulosclerosis. *Kidney Int* 2011; **80**: 483–492.
7. Möller CC, Wei C, Altintas MM *et al.* Induction of TRPC6 channel in acquired forms of proteinuric kidney disease. *J Am Soc Nephrol* 2007; **18**: 29–36.
8. Giardino L, Armelloni S, Corbelli A *et al.* Podocyte glutamatergic signaling contributes to the function of the glomerular filtration barrier. *J Am Soc Nephrol* 2009; **20**: 1929–1940.
9. Rastaldi MP, Armelloni S, Berra S *et al.* Glomerular podocytes contain neuron-like functional synaptic vesicles. *FASEB J* 2006; **20**: 976–978.
10. Puliti A, Rossi PIA, Caridi G *et al.* Albuminuria and glomerular damage in mice lacking the metabotropic glutamate receptor type 1. *Am J Pathol* 2011; **178**: 1257–1269.

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Defective neutrophil rolling and transmigration in acute uremia

Jana Pindjakova[1] and Matthew D. Griffin[1]

Circulating neutrophils are essential for innate immunity and undergo rapid, stepwise adhesion to and transmigration through the endothelium following tissue injury and microbial invasion. Neutrophil dysfunction may contribute to morbidity and mortality in acute kidney injury but has not frequently been studied at a mechanistic level. Rossaint *et al.* provide experimental evidence in mice and humans that acute uremia causes discrete intracellular signaling abnormalities that interfere with specific stages of neutrophil trafficking during inflammation.

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Bone marrow-derived neutrophils represent the most abundant circulating leukocyte population and are rapidly recruited to sites of tissue injury and microbial invasion. They play an essential, non-redundant role in wound healing and in the innate immune response to bacterial and fungal infection, as evidenced by the severity of neutrophil-specific inherited disorders such as chronic granulomatous disease.[1] Neutrophil effector functions include phagocytosis and the triggered release of a broad range of bioactive products, including reactive oxygen species, antimicrobial peptides, enzymes, cytokines, and chemokines—many of which are highly destructive of microbes and surrounding tissue alike.[1] Although frequently perceived as 'blunt instruments' in terms of immunological specificity, neutrophils are, in fact, highly regulated, interact extensively with other innate and adaptive immune cells, and participate actively in inflammatory resolution and tissue repair.[1,2]

It has been appreciated for some time that end-stage renal disease is associated

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